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Research Article

Polychlorinated Biphenyls and Leukocyte Telomere Length: An Analysis of NHANES 1999–2002

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ABSTRACT

Polychlorinated biphenyls (PCBs) induce the expression of the proto-oncogene c-myc which has a role in cellular growth and proliferation programs. The c-myc up-regulates the telomerase reverse transcriptase which adds the telomeres repeating sequences to the chromosomal ends to compensate for the progressive loss of telomeric sequence. We performed multivariate linear regression to analyze the association of PCBs, polychlorinated dibenzo-p-dioxins, and 1,2,3,4,6,7,8-heptachlorodibenzofuran with leukocyte telomere length (LTL) in the adult population (n = 2413) of the National Health and Nutrition Examination Survey 1999–2002. LTL was natural log-transformed and the results were re-transformed and presented as percent differences. Individuals in the 3rd and 4th quartiles of the sum of PCBs were associated with 8.33% (95% CI: 4.08–13.88) and 11.63% (95% CI: 6.18–17.35) longer LTLs, respectively, compared with the lowest quartile, with evidence of a dose–response relationship (p-trend < 0.01). The association of the sum PCBs with longer LTL was found in both sexes. Additionally, 1,2,3,4,6,7,8-heptachlorodibenzofuran and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin were associated with longer LTL. The age independent association between longer LTL and environmental exposures to PCBs, 1,2,3,4,6,7,8-heptachlorodibenzofuran and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin may support a role as tumor promoter of these compounds. Further studies to evaluate the effect of these compounds on LTL are needed to more fully understand the implications of our finding.

1. Introduction

Persistent organic pollutants (POPs) are lipophilic stable chemicals that bio-accumulate in adipose tissue of living organism (Van den Berg et al., 1998). Most POPs, such as polychlorinated biphenyls (PCBs), have already been banned in many countries. However, because of their persistence, along with the fact that production and release into the global environment is still occurring in some countries they are still present in the environment and food supply (Lauby-Secretan et al., 2013). Therefore, they are continually posing health hazards. POPs such as PCBs, polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) are associated with increased risks for various chronic diseases and cancer (ATSDR, 2000, 2002a, 2002b). There are 209 possible PCB congeners with different numbers and positions of substituted chlorine atoms on the aromatic rings. PCBs can act as endocrine-disrupting agents and both estrogenic and anti-estrogenic effects of PCBs have been reported in various *in vitro* and *in vivo* models. Non-planar PCBs have been reported to have weak estrogenic activity (Faroon et al., 2001), whereas, anti-estrogenic activity has been frequently reported in coplanar dioxin-like PCBs through aryl hydrocarbon receptor (AhR)-dependent mechanisms (Safe and Wormke, 2003; Oenga et al., 2004). The degree to which a particular coplanar dioxin-like PCBs congener act on the AhR is measured in Toxic Equivalents (TEQs), a comparison with a standard set to the highly toxic dioxin-like compound 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (Van den Berg et al., 1998; Van den Berg et al., 2006).

In vitro studies show that PCBs induce the expression of *c-myc* which has a role in cellular growth and proliferation programs (Dang, 2013). The proto-oncogene *c-myc* up-regulates the telomerase reverse transcriptase (TERT), which add the telomeres repeating hexanucleotide (TTAGGG) sequences to the chromosomal ends to compensate for the progressive loss of telomeric sequence, thus promoting chromosomal stability (Aubert and Lansdorp, 2008). With each cell replication, telomeres shorten and ultimately lead to apoptosis or permanent cell-cycle arrest. Absolute telomere length (TL) depends on an individual's age, cellular replicative history, and tissue type (Aubert and Lansdorp, 2008). Several studies have found that, independently of chronological age, shorter telomere length is associated with cardiovascular diseases (Haycock et al., 2014), diabetes (Zee et al., 2010), and mortality (Weischer et al., 2012). Germ cells, certain white blood cells, and cancer cells have active telomerase enzyme, which make them relatively long-lived compared to other cell types (Aubert and Lansdorp, 2008). Longer

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TL should allow for longer cellular survival, which increases the chance of accumulation of genetic mutations; therefore, longer TL could be associated with the possibility of accumulating cancer-promoting mutations (Noy, 2009). On the other hand, excessive telomere loss may lead to genomic instability and promote carcinogenesis (Blasco, 2005). Until now, the epidemiological evidence for associations between LTL and cancer has been inconsistent with results reporting positive, negative, or null associations and this inconsistency may, among others, be attributed to technical methodology (Cunningham et al., 2013), and to the fact that specific cancer types may have different effects on LTL (Gu and Wu, 2013). Shorter telomeres are associated with increased risk for several cancers, among them bladder, breast, ovaries, kidneys, head and neck, esophagus, stomach, and lung cancer (Wentzensen et al., 2011). However, the meta-analyses stratified by study designed conducted by Wentzensen et al. (Wentzensen et al., 2011) reported that the increased cancer risk with short telomeres was mainly driven by case–control studies, thus, suggestive of a possible effects of reverse causation in case–control studies where the cancer itself or the therapeutic procedures may affect telomere length. In prospective studies, long telomeres have been associated with an increased risk of several cancers such as melanoma (Han et al., 2009), lung cancer (Lan et al., 2013; Seow et al., 2014), non-Hodgkin lymphoma (Lan et al., 2009), pancreatic cancer (Lynch et al., 2013), and prostate cancer (Julin et al., 2015). Interestingly, in a 12 years follow-up of 792 Normative Aging Study participants, Hou and colleagues (Hou et al., 2015) reported age-related LTL attrition among those who developed prostate and other cancers. However, they observed a decelerating age-adjusted LTL attrition in cancer cases as they approached diagnosis with significant longer LTL within 4 years pre-diagnosis. The findings lead the authors to suggest that "telomere-elongating mechanisms in blood leukocytes may also be activated by cancer initiation, leading to LTL elongation early during cancer development." (Hou et al., 2015) Recently, in a small cross-sectional study conducted in 84 healthy Korean adult, low-dose exposure to POPs, including PCBs were associated with longer leukocyte telomere length (LTL) (Shin et al., 2010). In this study we investigated the potential association on blood level of POPs (PCBs, PCDDs, and PCDFs) with LTL in a nationally representative sample of the non-institutionalized civilian US adult population (20 years and older) who participated in the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2002.

2. Methods

2.1. Study Design and Population

NHANES is a cross-sectional, nationally representative survey of the non-institutionalized civilian population of the United States conducted annually by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC) (Johnson et al., 2013). NHANES uses a complex, multistage probability sampling design, with oversampling of certain subgroups. Participants complete household surveys that include questions about demographics and health history, and they provide blood and urine samples collected during the physical examinations at mobile exam centers. All procedures are approved by the NCHS Research Ethics Review Board (Protocol #98-12 http://www.cdc.gov/nchs/nhanes/irba98.htm), and all participants provide written informed consent. The grouping we used consisted of 2 cycles (1999–2000 and 2001–2002) that were combined using NCHS recommendations (Johnson et al., 2013).

2.2. Measurements of POPs in Serum

PCBs, PCDDs, and PCDFs were measured for a randomly selected subsample that included one-third of NHANES participants. The individual chemicals were measured by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (HRGS/ID-HRMS). NHANES provides congener concentrations both on a weight–weight basis and lipid adjusted using concentrations of serum total cholesterol and triglycerides; we used the latter in our analyses. Although 49 POPs were measured in both NHANES 1999–2000 and 2001–2002, to avoid bias in estimation among those below the limit of detection (LOD), we selected the 11 POPs for which at least more than 72% of study subjects had concentrations >LOD: 3 PCDDs [1,2,3,6,7,8-hxcdd (<LOD = 27.5%); 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (<LOD = 13.8%); and 1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin (<LOD = 20.3%)], 1 PCDFs [1,2,3,4,6,7,8-Heptachlorodibenzofuran ((<LOD = 27.8%)], 2 dioxin-like PCBs [PCB126 (<LOD = 24.0%) and PCB169 (<LOD = 22.9%)], 3 non-dioxin-like PCBs [PCB138 (<LOD = 25.6%), PCB153 (<LOD = 20.8%), and PCB180 (<LOD = 22.5%)].

Three PCB exposure metrics were calculated to reflect the biomechanism related to dioxins: (Faroon et al., 2001) *a*) the sum of all 5 PCB congeners ($\sum$PCBs); *b*) the sum of 3 non-dioxin-like PCBs (congeners 138, 153, and 180); and *c*)the sum of 2 dioxin-like PCBs (congeners 126, and 169). In a complementary analysis, we applied toxic equivalency factors (TEFs) published by the World Health Organization in 2005 to account for the relative toxicity and concentration of dioxin-like congeners (Van den Berg et al., 2006), and we summed the values to obtain the toxic equivalents (TEQs). Therefore, we will have: i) a toxic equivalent dioxin-like (TEQDL) representing the sum of the TEFs of the 3 PCDDs, the 1,2,3,4,6,7,8-heptachlorodibenzofuran, and PCB126 and PCB169; and ii) a toxic equivalent dioxin-like PCBs that is the TEF sum of PCB126 and PCB129. For compounds measures at or below the detection limit, the values were recorded as the detection limit divided by the square root of 2.

2.3. LTL Measurements

Briefly, aliquots of purified DNA, isolated from whole blood using the Puregene (D-50 K) kit protocol (Gentra Systems, Inc., Minneapolis, Minnesota), were provided by NCHS. The LTL assay was performed in the laboratory of Dr. Elizabeth Blackburn at the University of California, San Francisco, using the quantitative polymerase chain reaction method to measure LTL relative to standard reference DNA (also known as the T/S ratio) (Needham et al., 2013). Each sample was assayed 3 times on 3 different days. The samples were assayed on duplicate wells, resulting in 6 data points. Control DNA values were used to normalize between-run variability. Runs with more than 4 control DNA values falling outside 2.5 standard deviations from the mean for all assay runs were excluded from further analysis (<6% of runs). For each sample, any potential outliers were identified and excluded from the calculations (<2% of samples). The CDC conducted a quality control review before linking the LTL data to the NHANES public-use data files. The CDC Institutional Review Board provided human subject approval for this study. LTL was not normally distributed, thus it was natural log-transformed in our analyses.

2.4. Covariates

Models were adjusted for *a priori* factors based on previous literature demonstrating an association with LTL (Needham et al., 2013). These variables were age (years, continuous), age squared, sex, education (less than high-school, high school graduate, some college and above), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, multiracial, or other), alcohol consumption (amount consumed per week, categorized as no alcohol, 1–4 drinks/week, of >4 drinks/week), self-reported smoking status (current, former, or never smoker), serum cotinine (natural log-transformed), and body mass index. BMI was obtained from the physical examination and was calculated by dividing measured weight in kilograms by measured height in meters squared. Since inflammation is associated with telomere length (Rode et al., 2014), additional adjustment for c-reactive protein (a biomarker for inflammation) was used as a sensitivity analysis. PCBs act as endocrine-disrupting agents through sex hormone-related nuclear receptors (Safe, 2000), and hormonal regulation of telomere length is

described in the literature (Bayne and Liu, 2005). Thus, we further analyze the association of the sum of PCBs with LTL by gender stratification.

2.5. Statistical Analysis

All analyses were performed using the weights from the dioxin subsample as recommended by NCHS to account for the complex sampling design and non-response of NHANES and were calculated according to NHANES guidelines (Johnson et al., 2013). SAS 9.3 (SAS Institute, Cary, NC) was used for all statistical analyses and SAS-Callable SUDAAN 10 (Research Triangle Institute, Research Triangle Park, NC) was used to account for the NHANES complex sample design. All tests were two sided, and $p < 0.05$ was the level of significance. POPs were categorized as weighted quartiles based on the distribution of serum POP levels among the study population, resulting in approximately the same number of participants within each quartile. Evaluation of chemical outliers was performed by sampling weight as described by CDC NHANES tutorial (http://www.cdc.gov/nchs/tutorials/nhanes/Preparing/CleanRecode/Task3.htm). Exclusion of the POP outliers did not change the statistical significance of the results (data not shown).Therefore all the analyses are presented with the inclusion of the chemical outliers.

We used multivariate linear regression to calculate adjusted β-coefficients for the associations between natural log-transformed LTL and quartiles of POPs. Since our dependent variable, LTL, was log-transformed, the results were re-transformed by exponentiation of the β coefficients ($e^{\beta} \approx 1+\beta$) and presented as percent differences estimated by comparing each of the upper 3 quartiles to the lowest quartile; statistical tests for linear trends were conducted by modeling quartiles as an ordinal variable using integer values.

3. Results

The mean (standard error) LTL of the study population (n = 2431) was 1.06 (0.02). The weighted distributions of study population characteristics of the total sample are shown in Table 1). Women represented slightly over 51% of the total sample; the geometric mean age of the participants was approximately 43 years old, obesity prevalence was slightly higher of 29% and the prevalence of current smokers was 25.51%. Table 2 show the geometric mean (GM) and standard error (SE) of the serum POP concentrations among the participants in our study.

Table 3 shows the results of the multivariable linear regression. Briefly, in the adjusted model, the 3rd and 4th quartiles of the sum of PCBs (∑ PCBs) were associated with 8.33% (95% CI: 4.08–13.88) and 11.63% (95% CI: 6.18–18.53) longer LTLs, respectively, compared with the lowest quartile. There was evidence of dose–response relationship (p-value for trend <0.01). Similarly, the sum of non-dioxin-like PCBs, as well as the individual congeners PCB138, PCB153, PCB180 (Supplemental Table 1), were statistically significantly associated with longer LTL for the highest 3rd and 4th quartiles. The highest 3rd and 4th quartiles of the sum of dioxin-like PCBs compared to the lowest quartile were also associated with longer LTL with a dose–response relationship (Table 3). The same was found for the congener PCB169 (Supplemental Table 1), whereas only the highest quartile of PCB126 was associated with longer LTL (Supplemental Table 1).

Among the other compounds investigated, the highest quartile of the polychlorinated dibenzodioxin 1,2,3,4,6,7,8-heptachlorodibenzofuran was associated with longer LTL compared with the lowest, quartile (Table 3). There was a statically significant association of shorter LTL with age. For each year unit, LTL decreased of 0.80% (95% CI: −1.09 to −0.40) (data not shown).

No significant associations were observed between LTL and the sum of PCDD (Table 3), however the congener 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin) was associated with longer LTL (Supplemental Table 1). However, this association was not linear as indicated but the lack of statistical association between natural log-transformed 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin and LTL (Supplemental Table 2). Analyses stratified by sex, confirmed the association of ∑ PCBs, with longer LTL in both sexes (Supplemental Table 3). In complementary analyses using TEQ, the highest quartiles of TEQDL and the TEQPCBs, compared with the respective lowest quartiles, were associated with 11.63% (95% CI: 4.08–18.53) and 6.18 (95% CI: 1.01–11.63) longer LTL; both demonstrated a dose–response relationship (Table 4).

Complementary analyses using the natural log transformed compounds found a statistical significant association of the compound used in the primary analyses, except for 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (Supplemental Table 2). Sensitivity analyses including c-reactive protein measurements yielded results similar to those from the primary analyses (data not shown).

Table 1
Sample size and weighted characteristics of the NHANES 1999–2002 participants 20 years and older.

	ALL	
	n	Weighted
	2431	
Sex		
Men	1152	48.70 (1.11)
Women	1279	51.30 (1.11)
Age (Years), GM (SE)	2431	42.81 (0.52)
BMI(kg/m^2), GM (SE)	2431	27.23 (0.17)
Serum cotinine (ng/mL), GM (SE)	2431	0.68 (0.09)
Mean telomere length, GM (SE)	2431	1.03 (0.01)
Smoking status		
Current smoker, % (SE)	535	25.51 (1.18)
Former smoker, % (SE)	669	26.13 (1.35)
Never smoked, % (SE)	1227	48.37 (1.06)
Alcohol consumption		
No alcohol, % (SE)	850	30.58 (1.93)
1–4 drinks per week, % (SE)	1391	61.58 (2.26)
>4 drinks per week, % (SE)	190	7.84 (0.76)
Education level		
Less than high school % (SE)	821	21.77 (1.21)
Completed high school % (SE)	537	24.31 (1.61)
More than high school % (SE)	1073	53.92 (1.65)
Race/Ethnicity		
White (non-Hispanic) % (SE)	1219	72.25 (2.02)
Non-Hispanic Black % (SE)	425	10.10 (1.31)
Mexican-American % (SE)	576	6.96 (1.75)
Other Hispanic and other % (SE)	211	10.70 (1.78)

Table 2
Distribution of serum compound concentrations in the adults study population (20 years and older), NHANES 1999–2002.

	n	GM (SE)
∑ PCBs lipid adj (ng/g)	2175	82.13 (2.55)
∑ Non-dioxin-like lipid adj (ng/g)	2413	81.89 (2.54)
PCB138 lipid adj (ng/g)	2422	23.23 (0.73)
PCB153 lipid adj (ng/g)	2422	33.95 (1.06)
PCB180 lipid adj (ng/g)	2424	23.16 (0.76)
∑ Dioxin-like PCBs lipid adj (pg/g)	2202	38.31 (1.29)
PCB 126 lipid adj (pg/g)	2215	18.72 (0.69)
PCB 169 lipid adj pg/g)	2216	16.19 (0.51)
∑ PCDDs lipid adj (pg/g)	2115	370.90 (14.02)
1,2,3,6,7,8-hxcdd. lipid adj (pg/g)	2215	21.45 (1.15)
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin lipid adj (pg/g)	2203	37.78 (1.57)
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin lipid adj (pg/g)	2175	296.91 (10.90)
PCDF		
1,2,3,4,6,7,8-Heptachlorodibenzofuran (pg/g)	2090	7.70 (0.29)
TEQDL lipid adj (pg/g)	1976	6.01 (0.23)
TEQPCB lipid adj (pg/g)	2202	2.53 (0.09)

Table 3
Percent difference (95% CI)* in leukocyte telomere length by POP exposure, NHANES 1999–2002.*

∑ PCBs	N = 2175
Q1 ≤ 45.70 ng/g	Referent
Q2 45.71–81.00 ng/g	3.05 (−1.00, 6.18)
Q3 81.01–142.79 ng/g	8.33 (4.08–13.88)
Q4 > 142.80 ng/g	11.63 (6.18–17.35)
p trend	<0.001
R-square	0.194
∑ Non-dioxin-like (PCB 138 + PCB 153 + PCB 180)	N = 2413
Q1 ≤ 45.65 ng/g	Referent
Q2 45.66–80.95 ng/g	3.05 (−1.00–6.18)
Q3 80.96–143.99 ng/g	8.33 (4.08–13.88)
Q4 > 143.99 ng/g	11.63 (6.18–18.53)
p trend	<0.001
R-square	0.196
∑ Dioxin-like PCB (PCB 126 + PCB 169)	N = 2202
Q1 ≤ 21.93 pg/g	Referent
Q2 21.94–38.58 pg/g	3.05 (−1.00–6.18)
Q3 38.59–64.43 pg/g	6.18 (2.02–10.52)
Q4 > 64.43 pg/g	9.42 (3.05–16.18)
p trend	0.01
R-square	0.193
∑ PCDDs	N = 2115
Q1 ≤ 212.42 pg/g	Referent
Q2 212.43–359.62 pg/g	−1.00 (−6.76–4.08)
Q3 359.63–628.46 pg/g	2.02 (−2.96–7.25)
Q4 > 628.46 pg/g	3.05 (−3.92–10.52)
p trend	0.02
R-square	0.187
PCDF	
1,2,3,4,6,7,8-Heptachlorodibenzofuran (pg/g)	N = 2090
Q1 ≤ 4.62 pg/g	Referent
Q2 4.63–7.88 pg/g	5.13 (1.01–9.42)
Q3 7.89–12.46 pg/g	2.02 (−1.98–6.18)
Q4 > 12.46 pg/g	7.25 (3.05–11.63)
p trend	0.01
R-square	0.195

* Adjusted for age (years, continuous), age squared, education (less than high-school, high school graduate, some college and above), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, multiracial, or other), alcohol consumption, self-reported smoking status (current, former, or never smoker), serum cotinine (natural log-transformed),BMI and sex.

Table 4
Percent difference (95% CI) in leukocyte telomere length by TEQDL and TEQ PCBs exposure, National Health and Nutrition Examination Survey, 1999–2002*.

TEQDL	N = 1976
Q1 ≤ 3.61	Referent
Q2 3.62–6.17	4.08 (−1.00–9.42)
Q3 6.18–9.94	5.13 (−1.00–10.52)
Q4 > 9.94	11.63 (4.08–18.53)
p trend	0.01
R-square	0.206
TEQPCBs	N = 2202
Q1 ≤ 1.33	Referent
Q2 1.34–2.59	−1.00 (−4.88–3.05)
Q3 2.60–4.47	4.08 (−0.50–8.33)
Q4 > 4.47	6.18 (1.01–11.63)
p trend	0.01
R-square	0.191

* Adjusted for age (years, continuous), age squared, education (less than high-school, high school graduate, some college and above), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, multiracial, or other), alcohol consumption, self-reported smoking status (current, former, or never smoker), serum cotinine (natural log-transformed),BMI and sex.

4. Discussion

In this study, we found that ∑ PCBs and the individual congeners as well as the 1,2,3,4,6,7,8-heptachlorodibenzofuran and the 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin were associated with longer LTL. Our results are consistent with those reported by a study restricted to the NHANES 2001–2002 cycle dataset. Mitro and colleagues (Mitro et al., 2015) reported an association of dioxin-like PCBs (PCB126 and PCB169) and toxic equivalency (TEQ) (comprised of 7 dioxins, 9 furans, 2 non-ortho-substituted PCBs, and 6 mono-ortho-substituted PCBs) with longer LTL. The authors, also, reported an association of non-dioxin-like PCBs (comprised of 10 non-dioxin-like PCB congeners) with longer LTL; however, non-dioxin-like PCBs was not associated with longer LTL after adjustment for dioxin-like PCBs (Mitro et al., 2015). In our analyses, non-dioxin-like PCBs was still associated with longer LTL after adjustment for natural log-transformed sum of dioxin-like PCBs or TEQ (data not shown). The discrepancy with our finding of an association of non-dioxin-like PCBs to longer LTL may be due to sample size and the numbers of POP compounds used. We combined two NHANES cycle, thus increasing the sample size. Also, we limited our analyses only to compounds that were measured at or above the LOD in 75% of the sample, whereas Mitro et al. (Mitro et al., 2015) used PCBs congeners and dioxin and furans compounds that were detected at or above LOD in 50% of samples. There could, also, be difference in independent variables entered in the models. For example, Mitro et al. (Mitro et al., 2015) used, as independent variables, self-reported cancer and the percentage of white cell populations. However, adding to our analyses self-reported cancer as independent variable did not change the statistically significant association that we reported (data not shown).

Our results on the association between PCBs and longer LTL are consistent with the findings reported by Shin and colleagues (Shin et al., 2010) in a cross-sectional study consisting of 84 healthy Korean individuals (33 men and 51 women) with a mean age of 56 years (range 42–69 years). The authors reported a positive association of several PCBs — PCB99, PCB153, PCB180, PCB183 and PCB187 — with longer LTL (Shin et al., 2010).

In their evaluation on polychlorinated dibenzo-*para*-dioxins and polychlorinated dibenzofurans, the International Agency for Research on Cancer (IARC) classified 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) as carcinogenic to humans (IARC group 1 classification) but other polychlorinated dibenzo-p-dioxins, the nonchlorinated dibenzo-p-dioxin, and polychlorinated dibenzofurans were evaluated as not classifiable as to their carcinogenicity to humans (group 3) (IARC, 1997). PCBs are classified as probable carcinogens by the US Environmental Protection Agency (EPA) (USEPA, 1997). Recently, the IARC concluded that "there is *sufficient evidence* in humans for the carcinogenicity of polychlorinated biphenyls (PCBs). PCBs cause malignant melanoma. Positive associations have been observed for non-Hodgkin lymphoma and cancer of the breast." (IARC, 2015).

Furthermore, PCB126 and PCB169 were classified by IARC as a human carcinogen (IARC group 1 classification) (IARC, 2015).

A recent systematic review and meta-analysis found evidence of a role of PCBs in the development of non-Hodgkin Lymphoma (NHL) (Zani et al., 2013). Freeman and Kohles (Freeman and Kohles, 2012) suggested a causal relationship between NHL and PCB exposure based on the Bradford-Hill criteria. Kramer and colleagues (Kramer et al., 2012), in their analyses of the scientific literature, concluded that the weight of evidence was supportive of a causal role of PCBs in NHL; a biologically plausible explanation of this relationship is the immunosuppressive and inflammatory effects of PCBs.

A prospective cohort study with 107 cases reported a dose–response relationship between quartiles of increasing TL and risk of NHL (OR = 3.6; 95% CI: 1.4–8.9) (Lan et al., 2009). This association suggested that individuals with longer telomeres have an elevated NHL risk.

One may expect that "from a biologic perspective, longer telomere length should allow for longer cellular survival, the accumulation of genetic mutations, and the possibility of accumulating potentially cancer promoting mutations"; (Noy, 2009) therefore, our findings of the association of PCBs with longer LTL may suggest a plausible mechanistic

explanation for PCBs role in NHL development. PCBs may act to preserve LTL and possibly act as tumor promoters.

Studies in mice and rats demonstrated that PCBs are efficient tumor promoters when administered for extended periods of time following an initiating agent (Faroon et al., 2001; Safe, 1994). Several experimental systems associated activation of the proto-oncogene *c-myc* with cellular growth and proliferation programs (Dang, 2013) and thus an important feature of cancer initiation and maintenance (Gabay et al., 2014). Moreover, expression of TERT and telomerase activity is induced by *c-myc*; (Greenberg et al., 1999) therefore, it may be biologically plausible that PCBs may contribute to telomere length maintenance or lessening the loss by attrition by inducing *c-myc* expression. Gribaldo and colleagues (Gribaldo et al., 1998) showed that PCB138-exposed mouse fibroblast T3-L1 cell lines cultured in serum-free media overexpressed oncogenes that are involved in cell-cycle progression and proliferation, among them *c-myc*. Exposure of 3T3-L1 cells with PCB169 or PCB126 showed increased expression of *c-myc* in media containing serum (Gribaldo et al., 1998). Ghosh and colleagues (Ghosh et al., 2007) in an *in vitro* experiment using human liver (HepG2) cells, showed that chronic exposure of these cells with PCB153 overexpressed *c-myc* protein compared to short-term exposure. Another underlying mechanism for PCBs contribution to telomere length maintenance may be through activation of the NF-κB which upregulate the human telomerase (Akiyama et al., 2002; Zuo et al., 2011). Kwon and colleagues (Kwon et al., 2002) reported induction of NF-κB of human leukemic mast cell (HLMC) line exposed to PCB153.

There are few *in vitro* studies that investigate telomere length with PCBs exposure. Immortalized human skin keratinocytes (HaCats) treated with non-dioxin-like PCB28 and PCB52 for up to 48 days demonstrated a significant shortening of mean telomere length from days 18–48 (Senthilkumar et al., 2011). In another *in vitro* study, HaCat cells and normal human foreskin keratinocytes (NFK) were treated with PCB153 for up to 48 and 24 days, respectively (Senthilkumar et al., 2012). Shortening of telomere length was found only in HaCat cells, but not in NFK cells (Senthilkumar et al., 2012). These results may suggest that PCB action on telomere length could be cell type specific. There are no studies on telomerase activity induced by HPCDF or HXCDD. However, in a study using human choriocarcinoma BeWo cell line exposure to TCDD induced human telomerase both by direct and indirect induction of c-myc (Sarkar et al., 2006). Therefore, it may be feasible that also HPCDF and HXCDD may act in a similar fashion.

Although the strength of our study is that it is based on a nationally representative survey, the main study limitation is the cross-sectional design of NHANES; therefore, we cannot infer a temporal, causal association. There could also be confounding by other unmeasured variables, such as dietary components (Tiainen et al., 2012), proportion of different leukocyte subtypes (Lin et al., 2010). Furthermore, this study focused on the association between POPs and LTL, but people are exposed to a wide-range of chemicals including heavy metals and other pesticides that may have had a confounding effect on the associations we observed.

In conclusion, in this largest study to date of exposure to POPs and telomere length, we found an age-independent association between environmental exposures to PCBs,1,2,3,4,6,7,8-heptachlorodibenzofuran and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin and with LTL after adjustment for potential confounders. Further studies, such as well-designed prospective studies to evaluate the effect of PCBs on LTL, particularly with morbidity outcomes, are needed to more fully understand the implications of the findings of this study.

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None.

Conflict of Interest

The authors report no conflict of interest. Author Contributions.

Study concept and design by FS; data acquisition by FS; data analysis and interpretation by FS and MCB; manuscript drafting by FS; critical revision of the manuscript for important intellectual content by FS and MCB; study supervision by FS.

Disclaimer

The findings and conclusion in this report are those of the author and do not necessarily represent the views of CDC/ATSDR.

IRB approval

CDC/ATSDR has determined that our research did not meet the criteria for human research as per federal regulation and therefore did not require review.

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Appendix A. Supplementary data

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